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Chlorobenzene-Impaired Lindane Metabolism and the Effect of Pretreatment with Chlorobenzene, Lindane, or Chlorobenzene Plus Lindane

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16. ABSTRACT

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CHLOROBENZENE-IMPAIRED LINDANE METABOLISM AND THE EFFECT OF PRETREATMENT WITH CHLOROBENZENE, LINDANE, OR CHLOROBENZENE PLUS LINDANE

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The storage and metabolism of lindane (γ-HCH) was studied in the female rat after the administration of a hepatotoxic dose of chlorobenzene. Impaired lindane metabolism was observed following a challenge dose of 1.12 g chlorobenzene/kg. The data indicated that a hepatotoxic dose of chlorobenzene (CB) selectively impaired certain pathways, such as dehydrochlorination and the direct hydroxylation of lindane, to a greater extent than others, such as the dehydrogenation and dechlorination of lindane. Pretreatment with a subtoxic level of chlorobenzene produced: (1) significant increases in the dehydrogenation of lindane, (2) significant increase in the excretion of conjugated metabolites, (3) significant increases in the excretion of metabolites derived from the dehydrogenation of lindane through hexachlorocylohexene, γ-HCCH, (4) significant improvement in the excretion of metabolites derived from CB-impaired dehydrochlorination of lindane as well as from the CB-impaired hydroxylation of lindane, and (5) significant reduction in the level of unaltered lindane stored in the adipose tissue. Repeated pretreatment with a subtoxic level of chlorobenzene offered significant protection against the reduction in linaane metabolism produced by the single hepatotoxic dose of chlorobenzene. Pretreatment with γ-HCH alone was not as effective against the hepatotoxic effect of CB on lindane metabolism.

INTRODUCTION

Since environmental toxicants are frequently encountered as mixtures of chemicals, toxic components of these mixtures are likely candidates for chemicobiological interactions if they compete for the same metabolic

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This paper has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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pathways, induce or inhibit hepatic enzymes, and/or are likely to coexist in the environment as a result of manufacturing, processing, agricultural, and/or industrial use operations. Two such toxicants are the organochlorine insecticide hexachlorocyclohexane (lindane) and monochlorobenzene because (1) both induce hepatic enzymes (Carlson and Tardiff, 1976; Oesch et al., 1973; Kolmodin-Hedman et al., 1971; Den Tonkelaar and Van Esch, 1974; Mikol et al., 1980); (2) both chemicals utilize metabolic pathways such as UDPglucuronyltransferase, sulfotransferase, and glutathione transferase (Jerina and Daly, 1974; Kurihara et al., 1979; Chadwick et al., 1981); (3) both are produced by the chlorination of benzene (Colson, 1979); (4) both have been identified in environmental media from the same site (U.S. Environmental Protection Agency, 1982); and (5) both are toxic chemicals (Brodie et al., 1971; Reuber, 1979). Consequently, the effect of exposure to either chemical on the disposition and metabolism of the other was investigated. This study was designed to determine whether pretreatment with subtoxic levels of chlorobenzene and lindane, alone or in combination, modified the alteration in lindane metabolism induced by a hepatotoxic dose of chlorobenzene. The effect of lindane pretreatment on the disposition, biotransformation, and toxicity of chlorobenzene will be presented in a separate paper.

MATERIALS AND METHODS

Chlorobenzene (99%) was obtained from Aldrich Chemical Company, Inc., Milwaukee, Wis. [U-^{14}C]Chlorobenzene (specific activity 10 mCi/mmol, radiochemical purity 96%) was obtained from Amersham, Arlington Heights, Ill. Lindane (>99% γ-hexachlorocyclohexane) was obtained from the Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park, N.C. [U-^{14}C]Lindane (specific activity 48 mCi/mmol, radiochemical purity 98%) was obtained from Amersham, Arlington Heights, Ill.

In preliminary work, 6 young adult female Sprague-Dawley rats (CD strain, 220–300 g) were dosed perorally (po) with 10.8 mg lindane (containing 4.5 μCi [U-^{14}C]lindane)/kg. Concurrently, 2 animals per group were injected ip with either none, 112 mg, or 1.12 g chlorobenzene/kg. This work was conducted to determine whether chlorobenzene exposure had a detectable effect on lindane metabolism and to establish which pathways were affected most.

Next, 30 female weanling Sprague-Dawley rats, individually housed in metabolism cages, were randomly assigned to 1 of 5 treatment groups. One group of 6 animals was dosed po with only the peanut-oil vehicle throughout the study and served as the negative controls (NC) for the assay of *in vitro* enzyme activity. The remaining animals were part of a 2 X 2 factorial-designed experiment involving lindane and chlorobenzene. One group of 6 rats was dosed po with the vehicle for 7 d and with 1.12

g chlorobenzene (containing 18.7 μCi [U-^{14}C]chlorobenzene + 20 mg lindane)/kg on d 8. These rats were designated the positive controls (PC) and were used to compare the effect of pretreatment on CB-impaired lindane metabolism. The remaining rats received daily po injections of peanut-oil solutions of either 20 mg lindane/kg (γ-HCH), 300 mg chlorobenzene/kg (CB), or 300 mg chlorobenzene + 20 mg lindane/kg (CB + γ-HCH) for 7 d. On d 7, urine was collected for 24 h. On d 8 the γ-HCH, CB, and CB + γ-HCH groups were dosed po with 1.12 g chlorobenzene (containing 18.7 μCi [U-^{14}C]chlorobenzene + 20 mg lindane)/kg. The pretreatment level of lindane is about one-fifth the LD50 (Gaines, 1969) and has not produced overt signs of toxicity when previously employed in this lab. The pretreatment level of chlorobenzene was less than the nontoxic 400-mg/kg dose used by Carlson and Tardiff (1976) to study the effect of chlorobenzene on the metabolism of foreign organic compounds. The challenge dose of 1.12 g chlorobenzene was based on the 10-mmol/kg chlorobenzene dose used by Oesch and co-workers (1973) to produce centrilobular hepatocellular necrosis in rats. Following treatment on d 8, the animals were transferred to animal containment chambers (Plas-Labs, Lansing, Mich.), and urine, feces, and expired air were collected for 24 h. The animals were then transferred to their former cages, where they remained until sacrifice 24 h later.

Urine was extracted and partitioned as previously described (Chadwick et al., 1978). Urine extracts were analyzed for lindane metabolites on a Tracor Model MT-220 gas chromatograph equipped with a ^{63}Ni electron-capture detector and linearizer. The columns used consisted of 183 × 0.63 cm OD glass U-tubes packed with either 1.5% OV-17 + 1.95% QF-1 on 80/100-mesh Gas Chrom Q (Applied Science Laboratories, Inc., State College, Pa.) or 5% EGS + 1% H_2SO_4 on 90/100-mesh Anakrom A (Analabs, North Haven, Conn.). Both columns were maintained isothermally at 170°C. Inet and detector temperatures were 200 and 270°C, respectively. The 5% methane/argon carrier gas was regulated at 55 cm^3/min. Radioactivity was analyzed in a Packard Tri-Carb Model 3380 liquid scintillation spectrometer, using a scintillation solution containing 500 ml toluene, 500 ml cellosolve, 5.0 g 2,5-diphenyloxazole (PPO), and 50 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP).

At necropsy, fat samples were excised and extracted with hexane, and the extracts were analyzed by electron-capture gas chromatography for lindane content. Lipid was determined by evaporating the solvent and weighing the residue. Liver samples were homogenized and the enzyme activity involved in the dehydrogenation and dechlorination of lindane was determined (Chadwick et al., 1975).

Analysis of variance using planned comparisons and Student's *t*-test (Snedecor and Cochran, 1967) were used as aids in the interpretation of the data from this study. Comparisons were considered significantly different at $p < 0.05$.

RESULTS

Abbreviations used in the following sections include: Pc, positive control; NC, negative control; CB, chlorobenzene; γ-HCH, γ-hexachlorocyclohexane (lindane); 2,4,6-TCP, 2,4,6-trichlorophenol; 2,3,5-TCP, 2,3,5-trichlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol; 2,3,4,6-TTCP, 2,3,4,6-tetrachlorophenol; 2,3,4,5-TTCP, 2,3,4,5-tetrachlorophenol; 2,3,4,6-TCCOL, 2,3,4,6-tetrachloro-2-cyclohexen-1-ol; 2,4,5,6-TCCOL, 2,4,5,6-tetrachloro-2-cyclohexen-1-ol; PCCOL, 2,3,4,5,6-pentachloro-2-cyclohexen-1-ol; γ-TCCH, γ-3,4,5,6-tetrachlorocyclohex-1-ene; γ-HCCH, γ-1,2,3,4,5,6-hexachlorocyclohexene; PCCH, 2,3,4,5,6-pentachlorocyclohexene; TCPMA, trichlorophenylmercapturic acid; DCPMA, dichlorophenylmercapturic acid; CPMA, chlorophenylmercapturic acid.

Data from the preliminary study presented in Table 1 indicated that treatment with 1.12 g chlorobenzene impaired the biotransformation of lindane and/or the excretion of its metabolites. Excretion of the chlorophenol metabolites was more affected than that of the neutral metabolites, with the chlorobenzene treatment decreasing urinary chlorophenol content to about one-fifth that excreted by the controls. There were no significant difference in body weight, liver weight, kidney weight, or lung weight between the treated animals.

However, the treatment significantly elevated two key enzymatic pathways (Table 2). Both dehydrogenation, the rate-limiting step in the metabolism of lindane (Chadwick et al., 1975), and dechlorination were significantly induced in treated rats when compared to the untreated or negative controls (NC). Furthermore, rats pretreated with lindane (γ-HCH), chlorobenzene (CB), or the combination of both (CB + γ-HCH) had significantly higher dehydrogenase activity than the positive controls (PC). Moreover, the coadministration of chlorobenzene and lindane induced additive increases in the dehydrogenation of lindane.

The effect of treatment on the storage and excretion of lindane and eight metabolites is presented in Table 3. While there were no significant differences in the excretion of unconjugated metabolites, pretreatment with chlorobenzene induced a significant increase in the excretion of conjugated metabolites and a significant reduction in the stored level of unaltered lindane.

The effect of administering a hepatotoxic dose of chlorobenzene on the excretion of lindane and each of eight metabolites is presented in Figs. 1 and 2. In Fig. 1, the metabolite excretion of the positive controls and that of the chlorobenzene-pretreated rats, 24 h after treatment with a hepatotoxic dose of chlorobenzene, are compared. As indicated in the preliminary work (Table 1), treatment with the hepatotoxic dose of chlorobenzene, alone or in combination with γ-HCH, impaired the formation of lindane metabolites by the PC rats. However, repeated pretreatment with a subtoxic level of chlorobenzene for 7 d protected the animals from this

TABLE 1. Effect of Chlorobenzene on Excretion of Lindane-Derived Radioactivity[a]

Treatment[b]	Free neutrals[c]	Ratio[d]	Conjugated neutrals[e]	Ratio[d]	Free chlorophenols[f]	Ratio[d]	Conjugated chlorophenols[g]	Ratio
Controls	0.170 ± 0.010	1.0	2.60 ± 0.250	1.0	2.12 ± 0.285	1.0	5.44 ± 0.535	1.0
112 mg CB	0.145 ± 0.045	0.85	1.56 ± 0.430	0.60	1.20 ± 0.365	0.56	3.17 ± 0.970	0.58
1.12 g CB	0.105 ± 0.015	0.62	0.805 ± 0.365[h]	0.31	0.400 ± 0.140[h]	0.19	1.42 ± 0.450[h]	0.26

[a] Values are mean % of administered dose ± SE.
[b] Treatment is described in methods section.
[c] Radioactivity extracted with benzene from acid urine (pH = 2) that did not partition into 0.15 *N* NaOH.
[d] Ratio = % of administered dose from treated rats/% of administered dose from controls.
[e] Benzene-soluble radioactivity that did not partition into 0.15 *N* NaOH after hydrolysis of the residual urine from the preliminary extraction.
[f] Radioactivity extracted with benzene from acid urine (pH = 2) that partitioned into 0.15 *N* NaOH.
[g] Benzene-soluble radioactivity that did partition into 0.15 *N* NaOH after hydrolysis of the residual urine from the preliminary extraction.
[h] Values are significantly different from control at $p < 0.05$ using Student's *t*-test.

TABLE 2. Comparative *In Vitro* Enzyme Activity[a]

Treatment[b]	Dehydrogenation γ-HCH[c] → γ-HCCH[c]	Dechlorination γ-HCH[c] → γ-TCCH[c]
1. Negative control	0.137 ± 0.041	0.098 ± 0.013
2. Positive control	0.289 ± 0.032	0.145 ± 0.013
3. Chlorobenzene	0.407 ± 0.026	0.176 ± 0.026
4. Lindane	0.484 ± 0.077	0.222 ± 0.039
5. Chlorobenzene + lindane	0.721 ± 0.086	0.185 ± 0.022
Treatment comparison[d]	2,3,4,5 > 1 3,4,5 > 2 5 > 3,4	2,3,4,5 > 1

[a]Values are μg γ-HCCH/g liver·30 min (dehydrogenation) or μg γ-TCCH/g liver·30 min. (dechlorination), ±SE.
[b]Treatment is described in methods section.
[c]Abbreviations are defined in results section.
[d]Comparisons are significantly different at $p < 0.05$.

effect. The elevated excretion of PCCOL, 2,3,4,6-TTCP, and 2,3,4,5-TTCP in the chlorobenzene-pretreated rats is consistent with the increased dehydrogenase activity induced by chlorobenzene (Table 2), since this pathway is the rate-limiting step in the biotransformation of lindane to these metabolites (Fig. 3, Chadwick et al., 1975). Figure 2 presents the comparative metabolite excretion of the lindane-pretreated (Fig. 2a) and the chlorobenzene + lindane-pretreated rats (Fig. 2b) before and after administration of the challenge dose of chlorobenzene. Excretion of lindane metabolites, following the chlorobenzene treatment, was significantly higher from both

TABLE 3. Effect of Treatment on Storage and Excretion of Lindane and Metabolites[a]

	Urinary metabolites		
Treatment[b]	Free	Conjugated	Stored lindane
1. Positive controls	6.26 ± 0.620	53.4 ± 6.95	271 ± 24.3
2. Chlorobenzene	25.3 ± 10.4	214 ± 23.0	107 ± 21.4
3. Lindane	22.4 ± 6.73	353 ± 37.7	367 ± 79.8
4. Chlorobenzene + lindane	21.5 ± 3.46	480 ± 22.4	72.7 ± 12.2
Treatment comparisons[c]	NS[d]	3,4 > 1,2 2 > 1 4 > 3	 1 > 2 3 > 4

[a]Values for lindane plus 8 excreted metabolites are μg/24 h, ±SE, while values for stored lindane are ppm in lipid, ±SE.
[b]Treatment is described in methods section.
[c]Comparisons are significantly different at $p < 0.05$.
[d]Comparisons were not significant.

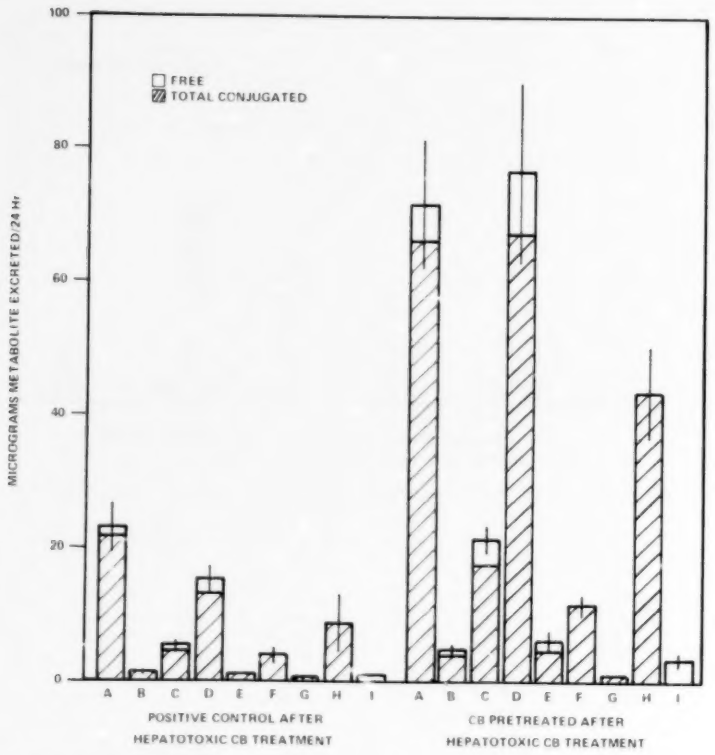

FIGURE 1. Effect of 1.12 g chlorobenzene/kg on the excretion of 8 lindane metabolites plus unaltered lindane by positive controls and chlorobenzene-pretreated rats. A, 2,4,6-TCP; B, 2,3,5-TCP; C, 2,4,5-TCP; D, 2,3,4,6-TTCP; E, 2,3,4,5-TTCP; F, 2,3,4,6-TCCOL; G, 2,4,5,6-TCCOL; H, PCCOL; I, γ-HCH. Each bar is the mean of six animals, with the shaded portion representing conjugated and the clear portion representing free metabolite. The vertical lines indicate the SE. Abbreviations were defined in results section.

these groups than from either the positive controls or the chlorobenzene-pretreated rats (Fig. 1, Table 3). However, the challenge dose of chlorobenzene significantly impaired the excretion of 5 of 6 metabolites from the lindane pretreated rats. Only the excretion of 2,3,4,6-TTCP and unaltered lindane were not significantly altered. Nevertheless, the decreases in the excretion of those metabolites derived from the dehydrogenation of lindane (Fig. 3), specifically PCCOL, 2,3,4,6-TTCP, and 2,3,4,5-TTCP were smaller than that observed for the other metabolites, and the overall impairment was proportionately less than that observed in the un-pretreated PC rats (Fig. 1). Significant induction of dehydrogenase activity in the lindane-pretreated rats (Table 2) may be responsible for this

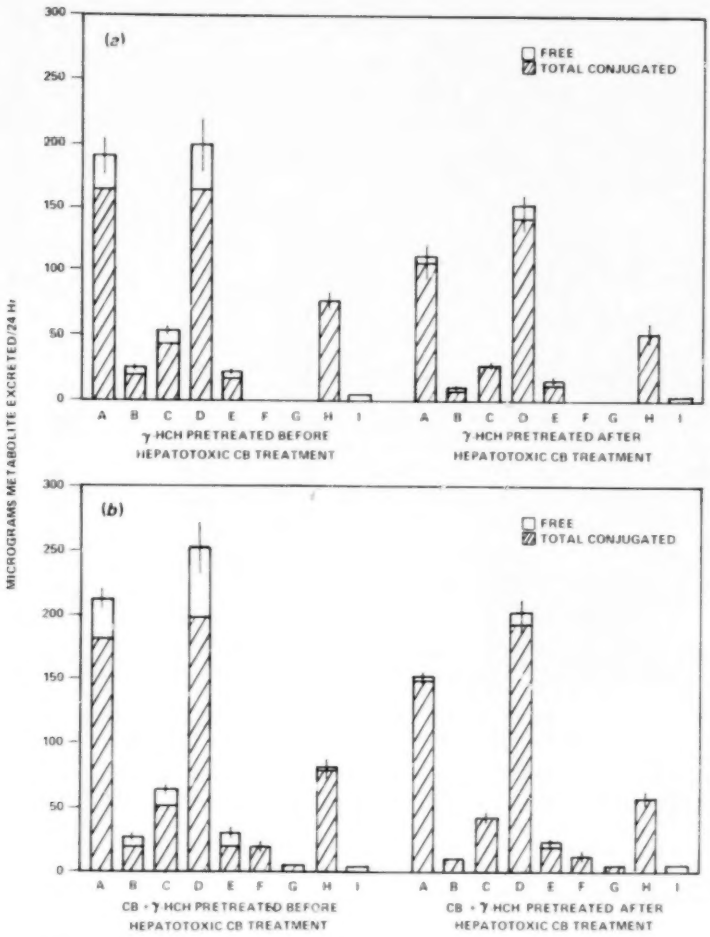

FIGURE 2. Effect of 1.12 g chlorobenzene/kg on the excretion of 8 lindane metabolites plus unaltered lindane by rats pretreated with (*a*) lindane or (*b*) chlorobenzene + lindane. A, 2,4,6-TCP; B, 2,3,5-TCP; C, 2,4,5-TCP; D, 2,3,4,6-TTCP; E, 2,3,4,5-TTCP; F, 2,3,4,6-TCCOL; G, 2,4,5,6-TCCOL; H, PCCOL; I, γ-HCH. Each bar is the mean of six animals, with the shaded portion representing conjugated and the clear portion representing free metabolite. The vertical lines indicate the SE. Abbreviations were defined in results section.

protective effect. The comparative excretion of 2,4,5,6-TCCOL and 2,3,4,6-TCCOL was not presented for the lindane-pretreated rats, since a number of samples were lost from the prechlorobenzene group. Coadministration of chlorobenzene and lindane for 7 d prior to the administration of a hepatoxic dose of chlorobenzene offered significantly greater protec-

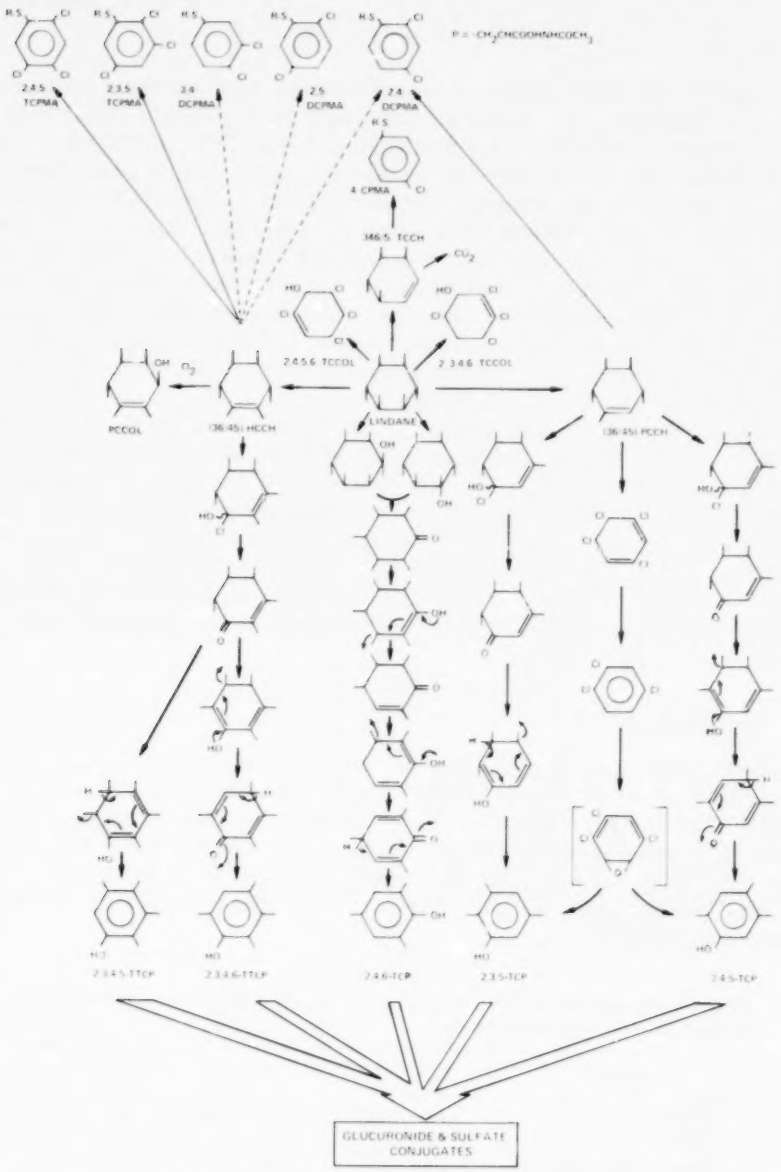

FIGURE 3. The biotransformation of lindane in rats. Where the configuration of the metabolites is known, this is indicated by a fractional notation, the numbers in the numerator denoting chlorine above and numbers in the denominator denoting chlorine below the plane of the ring. Abbreviations were defined in results section.

tion against the CB-impaired biotransformation and excretion of lindane and its metabolites. For example, there were no significant differences in the excretion of PCCOL, 2,4,5,6-TCCOL, 2,3,4,6-TTCP, and 2,3,4,5-TTCP 24 h before and 24 h after the challenge dose of 1.12 g chlorobenzene (Fig. 2). Moreover, the combined urinary content of 2,4,6-TCP, 2,3,5-TCP, 2,4,5-TCP, PCCOL, 2,3,4,6-TTCP, and 2,3,4,5-TTCP was elevated from 58% of normal for the lindane-pretreated animals to 70% for the chlorobenzene + lindane-pretreated rats. Again, the metabolites least affected by the hepatotoxic dose of chlorobenzene were those derived from the initial dehydrogenation of lindane, and this increased protection appears to be due to the additive effects of lindane and chlorobenzene on the dehydrogenase activity (Table 2).

From Fig. 1 it is apparent that the excretion of 2,4,6-, 2,3,5-, and 2,4,5-trichlorophenol was more seriously impaired by the challenge dose of 1.12 g chlorobenzene than was the excretion of the other 5 metabolites. Table 4 presents the percentage of the normal urinary content of these metabolites excreted by the PC, γ-HCH, and CB + γ-HCH groups. Pretreatment with CB + γ-HCH provides significant protection from the 3–5-fold reduction in the excretion of all three metabolites by the PC rats, whereas pretreatment with γ-HCH alone significantly improved only the excretion of 2,4,6-TCP.

DISCUSSION

Data from this study indicated that the treatment of rats with a hepatotoxic dose of chlorobenzene impaired the metabolism of a coadministered dose of the organochlorine insecticide, lindane. Together with a 3–5-fold decrease in the excretion of the lindane metabolites 2,4,6-, 2,3,5-, and 2,4,5-TCP were smaller but significant reductions in 2,3,4,6-TTCP, 2,3,4,6-TCCOL, and PCCOL, following treatment with 1.12 g chlorobenzene/kg (Fig. 1).

TABLE 4. Comparative Percent of Normal Urinary Trichlorophenol Content after a Hepatotoxic Dose of Chlorobenzene[a]

Treatment[b]	2,4,6-TCP[c]	2,3,5-TCP[c]	2,4,5-TCP[c]
1. Positive controls	34.7 ± 5.36[d]	20.5 ± 2.95[d]	39.2 ± 5.59[d]
2. Lindane	59.1 ± 6.93[e]	26.4 ± 3.20[d]	49.8 ± 6.80[d]
3. Chlorobenzene + lindane	72.4 ± 7.02[e]	38.8 ± 4.83[e]	67.1 ± 4.93[e]

[a] Values are % of excretion by rats unexposed to a hepatotoxic dose of chlorobenzene, ±SE.
[b] Treatment is described in methods section.
[c] Abbreviations were defined in results section.
[d–e] Values assigned the same superscript are not significantly different from one another at $p < 0.05$.

Pretreatment with CB alone or in combination with γ-HCH offered significant protection against the impaired excretion induced by the challenge dose of 1.12 g CB/kg (Figs. 1 and 2). Chlorobenzene, alone or in combination with lindane, significantly increased the dehydrogenation of lindane (Table 3), the excretion of total conjugates (Table 3), and the excretion of metabolites derived from the dehydrogenation of lindane through γ-HCCH (Figs. 1–3, Tables 2–4). These alterations were accompanied by significant improvement in the CB-impaired dehydrochlorination of lindane to 2,3,5-TCP and 2,4,5-TCP (Fig. 3, Table 4) and the CB-impaired hydroxylation of lindane through pentachlorocyclohexanone-gem-chlorohydrin to 2,4,6-TCP (Fig. 3, Table 4).

Pretreatment with lindane alone, on the other hand, was not as effective in countering the hepatotoxic effect of 1.12 g CB/kg on lindane metabolism. Although lindane induces its own metabolism (Chadwick and Freal, 1972) and despite significant induction of the dehydrogenation pathway (Table 2), only the excretion of 2,3,4,6-TTCP was not significantly impaired by 1.12 g CB/kg in the γ-HCH rats (Fig. 2). Moreover, γ-HCH pretreatment significantly improved only the direct hydroxylation of lindane to 2,4,6-TCP among the CB-impaired trichlorophenol metabolites (Table 4).

Results from this study indicate that chlorobenzene treatment markedly alters the metabolism of lindane in the rat. Moreover, the alterations are dose-dependent. A single hepatotoxic dose of CB impaired lindane metabolism, whereas repeated pretreatment with subtoxic levels of CB did not. Furthermore, repeated pretreatment with a subtoxic level of chlorobenzene offered significant protection against the reduction in lindane metabolism induced by the single hepatotoxic dose of chlorobenzene. Thus, predicting the potential for toxic interactions between environmental chemicals may require the study of combinations at different levels and for various periods of time in the whole animal.

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